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U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE		DATE: September 25, 1998
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EQ/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLN. NO. (if known): <b>09/147052</b>
INTERNATIONAL APPLICATION NO.: PCT/JP97/01084	INTERNATIONAL FILING DATE: March 28, 1997	PRIORITY DATE CLAIMED: March 29, 1996
TITLE OF INVENTION: NOVEL FUSION PROTEIN, GENE THEREOF, RECOMBINANT VECTOR BEARING THE GENE AND RECOMBINANT VIRUS AS WELL AS USE THEREOF		
APPLICANT(S) FOR DO/EQ/US: Shuji SAITO, Yoshinari TSUZAKI and Noboru YANAGIDA		
<p>Applicant hereby submits to the United States Designated/Elected Office (DO/EQ/US) the following items and other information:</p> <ol style="list-style-type: none"> <li><u>XX</u> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><u>XX</u> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><u>XX</u> This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).</li> <li><u>XX</u> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li><u>XX</u> A copy of the International Application as filed (35 U.S.C. 371(c)(2)):        a. <u>XX</u> is transmitted herewith (required only if not transmitted by the International Bureau).        b. <u>XX</u> has been transmitted by the International Bureau.        c. <u>XX</u> is not required, as the application was filed in the United States Receiving Office (RO/US)</li> <li><u>XX</u> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li><u>XX</u> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))        a. <u>XX</u> are transmitted herewith (required only if not transmitted by the International Bureau).        b. <u>XX</u> have been transmitted by the International Bureau.        c. <u>XX</u> have not been made; however, the time limit for making such amendments has NOT expired.        d. <u>XX</u> have not been made and will not be made.</li> <li><u>XX</u> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><u>XX</u> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li><u>XX</u> A translation of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol> <p>ITEMS 11. TO 16. BELOW CONCERN OTHER DOCUMENT(S) OR INFORMATION INCLUDED:</p> <ol style="list-style-type: none"> <li><u>XX</u> An Information Disclosure Statement under 37 CFR 1.97 and 1.98 together with the International Search Report and 7 references.</li> <li><u>XX</u> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><u>XX</u> A <b>FIRST</b> preliminary amendment.  <u>XX</u> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment</li> <li><u>XX</u> A substitute specification.</li> <li><u>XX</u> A change of power of attorney and/or address letter.</li> <li><u>XX</u> Other items or information: 8 sheets of drawings; translation of IPE report.</li> </ol>		

U.S. APPLICATION NO. (if known)	INTERNATIONAL APPLICATION NO. PCT/JP97/01084	DATE: September 25, 1998	
17. <u>X</u> The following fees are submitted: <b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b> Search Report has been prepared by the EPO or JPO:.....\$930.00 International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$720.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....\$790.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$1,070.00 International preliminary examination fee (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$ 98.00 ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 930.00		CALCULATIONS	PTO USE ONLY
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 <u>X</u> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
TOTAL	42 -20=	22	X \$ 22.00
INDEPENDENT	1 - 3=		X \$ 82.00
Multiple dependent claims(s) (if applicable)		+ \$270.00	\$ 270.00
TOTAL OF ABOVE CALCULATIONS =			\$1,814.00
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).			
SUBTOTAL =			\$1,814.00
Processing fee of \$130.00 for furnishing the English translation later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		+	
TOTAL NATIONAL FEE =			\$1,814.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +			
TOTAL FEES ENCLOSED =			\$1,814.00
		Amount to be: refunded	\$
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U.S. APPLICATION NO. (if known)	INTERNATIONAL APPLICATION NO. PCT/JP97/01084	DATE: September 25, 1998
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a. XX A check in the amount of \$1,814.00 to cover the above fees is enclosed.  
(This paper is filed in triplicate)

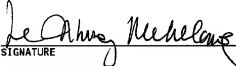
b.      Please charge my Deposit Account No. 01-2340 in the amount of \$      to cover the above fees.  
(A duplicate copy of this sheet is enclosed.)

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2340.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed to request that the application be restored to pending status.

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## DESCRIPTION

NOVEL FUSION PROTEIN, GENE THEREOF,  
RECOMBINANT VECTOR BEARING THE GENE  
AND RECOMBINANT VIRUS AS WELL AS USE THEREOF

## TECHNICAL FIELD

The present invention relates to a novel fusion polypeptide of a polypeptide having the antigenicity of Mycoplasma gallisepticum and a polypeptide derived from the outer membrane protein of herpes viruses, a hybrid DNA coding for the fusion polypeptide, and a recombinant Avipox virus bearing the hybrid DNA, as well as a vaccine using the recombinant Avipox virus.

## BACKGROUND ART

10           Mycoplasma gallisepticum (hereinafter sometimes abbreviated as MG) is a bacterium that causes reduction in an egg-laying rate and a hatching rate of eggs for poultry including chicken. This causative MG is widely spread all over the world so that a great deal of damage  
15 has been done to the poultry farming. For the prevention of MG, an inactivated vaccine or a live vaccine is currently utilized. However, the former live vaccine involves disadvantages of complicated inoculation procedures, short duration of immunity, expensive etc.  
20 The latter vaccine has such a defect that an unexpected

disease might be developed by use in combination with live vaccine for other disease. Another disadvantage is that MG agglutination reaction system, which makes rapid detection of MG infection possible, can not be used for both inactivated and live vaccines.

It is expected that a protein derived from MG such as its antigenic protein for preventing from MG infection would be produced by genetic engineering technology and utilized as a vaccine.

10           The production system of the antigenic protein of Mycoplasma gallisepticum using E. coli or yeast by means of genetic engineering (JPA 2-111795, etc.) encounters such problems that depending upon a protein to be expressed, the antigenic protein is only expressed in  
15 a less amount, proteins of host origin might be by-produced and intermingled, host-derived pyrogen is removed only with difficulty, etc. For these reasons, studies are still focused on a recombinant virus to prepare antigenic proteins or on a recombinant live  
20 vaccine.

          The expression of foreign genes using recombinant viruses, in most cases, genes of eucaryotes or viral genes are expressed. For this reason, addition or expression mode of sugar chains or the like is similar  
25 to the protein expression mechanism in infected cells. Thus, induction of an antibody titer to the expressed protein was relatively easy in vivo. However, genes of prokaryotes are rarely expressed in recombinant viruses.

Because of different expression mode between eukaryotes and prokaryotes, it was difficult to say that a specific antibody was effectively induced (Austen et al., Protein Targeting and Selection, Oxford Univ. Press (1991)).

5           Turning to MG, recombinant viruses in which a gene coding for the protein has been incorporated are known by JPA 5-824646 and JPA 7-133295, WO 94/23019, etc. In particular, WO 94/23019 reveals that when a recombinant virus capable of expressing the antigenic  
10 protein of MG having a viral membrane anchoring region, which is obtained by ligating the signal membrane anchoring portion of HN gene of New Castle disease virus (hereinafter abbreviated as NDV) with the antigenic gene of MG, is inoculated as a recombinant live vaccine, the  
15 antibody is induced more effectively than a recombinant virus capable of expressing the antigenic gene of MG alone.

          However, expression to such an extent is not always sufficient to achieve the desired effect as a  
20 vaccine.

          Therefore, it is the urgent need to find an improved method for higher recognition of the antigen in order to develop an effective vaccine against MG infections.

25           Outer membrane proteins other than NDV mentioned above are known also in the genus Herpesvirus, etc. With respect to glycoproteins B(gB), C(gC), D(gD), H(gH) and I(gI) of herpes simplex viruses; proteins gBh,

gCh, gDh, gHh and gIh of Marek's disease viruses (hereinafter often referred to as MDV) corresponding to herpes simplex virus glycoproteins gB, gC, gD, gH and gI and proteins of the genus Herpesvirus homologous to those  
5 proteins described above, etc., the nucleotide sequence and amino acid sequence of these proteins are known. It is also known that a part of these proteins induces neutralizing antibodies of herpes simplex viruses (Deluca et al., Virology, 122, 411-423 (1982)). It is further  
10 known that neutralizing antibodies can be induced by incorporating genes coding for these proteins into vaccinia viruses and expressing the genes (Blacklaws et al., Virology, 177, 727-736 (1990)).

However, investigations to make use of signal  
15 sequences of such outer membrane proteins of the genus Herpesvirus were hardly made so far.

#### DISCLOSURE OF THE INVENTION

Under the situation of the prior art stated above, the present inventors have made extensive studies  
20 to provide a recombinant virus capable of expressing a Mycoplasma antigenic protein having an enhanced infection prevention activity in large quantities, which allows a host to recognize the antigen highly efficiently. As a result, it has been found that by infecting to a host a  
25 recombinant Avipox virus, in which a hybrid DNA obtained by ligating a DNA of the outer membrane protein of the genus Herpesvirus with a DNA of the antigenic protein of

Mycoplasma has been inserted, the antigen recognizing ability of the host can be markedly improved. The present invention has thus been accomplished.

Accordingly, the present invention provides:

- 5           a fusion protein comprising a polypeptide having the antigenicity of Mycoplasma gallisepticum (hereinafter sometimes referred to as Mycoplasma-derived polypeptide) and a polypeptide derived from the outer membrane protein of a herpes virus (hereinafter sometimes referred to as Herpesvirus-derived polypeptide)
- 10           characterized in that the polypeptide derived from outer membrane protein is ligated with the polypeptide having the antigenicity of Mycoplasma gallisepticum at the N terminus thereof;
- 15           a hybrid DNA coding for the fusion protein;
- a recombinant Avipox virus in which the hybrid DNA has been incorporated; and,
- a live vaccine comprising the recombinant Avipox virus as an effective ingredient.

20   BRIEF DESCRIPTION OF THE DRAWINGS

            Fig. 1 is a drawing for explaining procedures for construction of pNZ40K-S.

            Fig. 2 is a drawing for explaining procedures for construction of pNZ40K-S.

- 25           Fig. 3 is a drawing for explaining procedures for construction of pNZ40K-S.

            Fig. 4 is a drawing for explaining procedures



for construction of pNZ40K-C.

Fig. 5 is a drawing for explaining procedures for construction of pNZ40K-C.

Fig. 6 is a drawing for explaining procedures  
5 for construction of pNZ40K-C.

Fig. 7 shows the results of Western blotting by which expression of TTM-1 polypeptide was confirmed.

Fig. 8 shows scores of the tracheal lesion caused.

#### 10 BEST MODE FOR PRACTICING THE INVENTION

##### Mycoplasma-derived polypeptides and genes therefor

In the present invention, the term Mycoplasma-derived polypeptides is used to mean the antigenic proteins that cause an antigen-antibody reaction with MG  
15 immune serum or MG infected serum and that are derived from MG. These polypeptides are not restricted to proteins per se that native Mycoplasma gallisepticum expresses, and may include modified polypeptides. For example, one or more amino acids of the polypeptides may  
20 be modified naturally or artificially in a conventional manner such as site-specific mutation, etc. (JPB 6-16709, etc.) through loss, addition, insertion, deletion, substitution, etc. Of course, the proteins, even after such modification, should contain the epitope showing the  
25 antigenicity. For determination of the epitope region, there are available known methods based on the peptide scanning technique such as the method of Geysen et al.

(J. Immunol. Meth., 102, 259-274 (1987)), the method of Hopp et al., (Proc. Natl. Acad. USA, 78, 3824-3828 (1981)), the method Chou et al. (Advances in Enzymology, 47, 145-148 (1987)), etc.

5           Specific examples of the peptides having the antigenicity include antigenic proteins disclosed in JPA 2-111795 (U.S. Patent Application Serial Nos. 359,779, 07/888,320 and 08/299,662), JPA 5-824646 (U.S. Patent No. 5,489,430), WO 94/23019 (U.S. Patent Application Serial

10 No. 08/525,742, JPA 6-521927) and proteins of Mycoplasma gallisepticum containing the amino acid sequences of those proteins. Of course, so long as the epitope is contained therein, a part of the peptides described above may also be usable.

15           Of these peptides, preferred are the polypeptide of about 40 kilodaltons (kd) described in JPA 5-824646, the polypeptide of about 66 kd encoded by TM-66 gene and the polypeptide of about 67 kd encoded by TM-67 gene described in JPA 5-521927, which are

20 designated as SEQ NO: 16 and SEQ NO: 27 therein.

          In the present invention, genes of the Mycoplasma-derived polypeptides bear DNA sequences coding for the polypeptide having the antigenicity of Mycoplasma gallisepticum described above. Such DNA can be obtained

25 by synthesis or acquired from wild bacteria belonging to Mycoplasma gallisepticum. Specific examples of such bacteria are strains R, S6, KP-13, PG31, etc. DNA may also be derived from MG isolated from wild strains.

These genes can also be modified by loss, addition, insertion, deletion, substitution, etc. in a conventional manner as described in Methods in Enzymology, etc.

#### Herpesvirus-derived polypeptides and genes thereof

5           The Herpesvirus-derived polypeptides in the present invention refer to polypeptides derived from proteins that construct an envelope of viruses belonging to the genus Herpesvirus. The Herpesvirus-derived polypeptides may not always be the full length of the

10 proteins. Where the polypeptides are used solely to be expressed on the surface of cell membranes function as fusion proteins, it is sufficient for the polypeptide to contain a membrane anchor and a signal sequence therein, and where the polypeptides are employed for secretion,

15 the polypeptides may contain only a signal sequence for that purpose. The outer membrane proteins may be either type I or type II of the outer membrane proteins. The signal sequence and the membrane anchoring sequence are both readily detectable by analyzing the amino acid

20 sequence in the hydrophobic peptide region at the carboxyl terminus or amino terminus thereof.

Specific examples of the outer membrane protein include gB, gC, gD, gH and gI which are glycoproteins of herpes simplex viruses, and gBh, gCh, gDh, gHh and gIh of

25 MDV corresponding to herpes simplex viruses glycoproteins gB, gC, gD, gH and gI, and proteins of the genus Herpesvirus homologous to the proteins described above.

Of course, polypeptides bearing the epitope other than the signal sequence of the outer membrane proteins may also be ligated with the aforesaid polypeptides having the antigenicity. By the ligation it is expected that the epitope will give the immunity to the living body in vivo.

In the present invention, the genes for the Mycoplasma-derived polypeptides contain DNA sequences coding for the Herpesvirus-derived polypeptides described above and such DNAs can be synthesized or acquired from naturally occurring herpes viruses. These genes may also be modified by loss, addition, insertion, deletion, substitution, etc. in a conventional manner as described in Methods in Enzymology, etc.

#### 15 Fusion protein and hybrid DNA

The fusion proteins of the present invention are obtained by incubating a recombinant Avipox virus inserted hybrid DNA, which will be later described, in culture cells such as chick embryo fibroblast cells (hereinafter referred to as CEF cells) or embryonated chorioallantoic membrane cells, etc.

The thus obtained fusion proteins can be employed as a component vaccine.

The hybrid DNA of the present invention comprises the gene for the Mycoplasma-derived polypeptide and the gene for the Herpesvirus-derived polypeptide, which are ligated with each other directly or via an

optional DNA sequence.

The hybrid DNA of the present invention can be produced in a conventional manner, for example, by a method in which the outer membrane protein and the antigenic protein of Mycoplasma gallisepticum are digested with restriction enzymes, respectively, and the resulting ligatable DNA fragment coding for the outer membrane protein of herpes viruses or for the signal sequence of the outer membrane protein is ligated with the resulting ligatable DNA fragment coding for the antigenic protein of Mycoplasma gallisepticum, using a ligase directly or via an appropriate linker.

Specific examples of the amino acid sequences for the fusion proteins of the present invention include SEQ NO: 2 and SEQ NO: 4. The sequence of the antigenic protein of 40 kilodaltons derived from Mycoplasma gallisepticum is found in amino acids 64-456 of SEQ NO: 2 and in amino acids 693-1086 of SEQ NO: 4. The signal sequence of outer membrane protein gB derived from MDV is found in amino acids 1-63 of SEQ NO: 2. In SEQ NO: 4, amino acids 1-672 correspond to almost the full length of outer membrane protein gB derived from MDV. Specific examples of nucleotide sequences of the hybrid DNAs coding for these fusion proteins are those shown by SEQ NO: 1 and SEQ NO: 3.

These fusion proteins and hybrid DNAs are given by way of examples but are not deemed to be limited thereto.

Recombinant Avipox virus

The recombinant Avipox virus of the present invention is a recombinant Avipox virus in which the aforesaid DNA or hybrid DNA has been inserted in the non-essential region. The recombinant Avipox virus of the present invention can be constructed in a conventional manner, e.g., by the method described in Japanese Patent Application Laid-Open No. 1-168279. That is, the non-essential region of Avipox virus is incorporated into a DNA fragment to construct a first recombinant vector.

As the non-essential region of Avipox virus which is used in the present invention, there are a TK gene region of quail pox virus, a TK region of turkey pox virus and DNA fragments described in JPA 1-168279, preferably a region which causes homologous recombination with EcoRI fragment of about 7.3 Kb, HindIII fragment of about 5.2 Kb, EcoRI-HindIII fragment of about 5.0 Kb, BamHI fragment of about 4.0 Kb, described in the patent specification supra.

Examples of the vector used in the present invention include plasmids such as pBR322, pBR325, pBR327, pBR328, pUC7, pUC8, pUC9, pUC18, pUC19, and the like; phages such as  $\lambda$  phage, M13 phage, etc.; cosmid such as pHC79 and the like.

The Avipox virus used in the present invention is not particularly limited so long as it is a virus infected to avian. Specific examples of such a virus include pigeon pox virus, fowl pox virus (hereafter

abbreviated as FPV), canary pox virus, turkey pox virus, preferably pigeon pox virus, FPV and turkey pox virus, more preferably pigeon pox virus and FPV. Specific examples of the most preferred Avipox virus include FPVs  
5 such as ATCC VR-251, ATCC VR-249, ATCC VR-250, ATCC VR-229, ATCC VR-288, Nishigahara strain, Shisui strain, CEVA strain and a viral strain among CEVA strain-derived viruses which forms a large plaque when infected to chick embryo fibroblast, and a virus such as NP strain (chick  
10 embryo-attenuated pigeon pox virus Nakano strain), etc. which is akin to FPV and used as a fowlpox live vaccine strain. These strains are commercially available and readily accessible.

Next, the hybrid DNA of the present invention  
15 is inserted into the non-essential region of the first recombinant vector described above to construct a second recombinant vector. In general, the hybrid DNA employed may have any nucleotide sequence, irrespective of synthetic or natural one, so long as the hybrid DNA  
20 effectively functions as a promoter in the system of transcription possessed by Avipox viruses. Accordingly, not only promoters inherent to Avipox viruses such as promoters for Avipox virus-derived genes coding for thymidine kinase but also DNAs derived from viruses other  
25 than Avipox viruses and DNAs derived from eukaryotes or prokaryotes may also be employed in the present invention, insofar as these substances meet the requirements described above. Specific examples of such

promoters include promoters for vaccinia viruses (hereinafter often referred to as VV) as described in Journal of Virology, 51, 662-669 (1984), more specifically, a promoter of VV gene coding for 7.5 K polypeptide, a promoter of VV gene coding for 19 K polypeptide, a promoter of VV gene coding for 42 K polypeptide, a promoter of VV gene coding for thymidine kinase, a promoter of VV gene coding for 28 K polypeptide, etc. Furthermore, there may be used a synthetic promoter obtained by modification of the Moss et al. method (J. Mol. Biol., 210, 49-76 and 771-784, 1989), Davidson's synthetic promoter, a promoter obtained by modifying a part of the Davidson's promoter through deletion or change in such a range that the promoter activity is not lost (e.g.,

TTTTTTTTTTTTTGGCATATAAATAATAATAAATACAATAATTAATTACGCGTAAAAA  
TTGAAAACTATTCTAATTATGCACTC,  
TTTTTTTTTTTTTTTTTTTTTGGCATATAAATAATAAATACAATAATTAATTACGCGT  
AAAAATTGAAAACTATTCTAATTATGCACTC etc.).

Further in view of easy detection of the recombinant virus, a marker gene such as a DNA coding for  $\beta$ -galactosidase may also be inserted.

The recombinant Avipox virus can be constructed by transfecting the second recombinant vector described above to animal culture cells, which has been previously infected with Avipox virus, and causing homologous recombination between the vector DNA and the viral genome DNA. The animal culture cells used herein can be any



cells, so long as Avipox can grow in the cells. Specific examples of such animal culture cells are CEF cells, embryonated egg chorioallantoic membrane cells, and the like.

- 5           The objective recombinant Avipox virus is isolated from the virus infected to host cells by plaque hybridization, etc.

#### Live vaccine

- The recombinant virus of the present invention  
10 constructed by the method described above can be inoculated to avian as a live vaccine for Mycoplasma gallisepticum infection.

- The live vaccine of the present invention is prepared by, e.g., the following method, though the  
15 process is not particularly limited thereto. The recombinant virus of the present invention is infected to cells in which the virus can grow (hereafter referred to as host cells). After the recombinant virus grows, the cells are recovered and homogenated. The homogenate is  
20 centrifuged to separate into the precipitates and the high titer supernatant containing the recombinant virus. The resulting supernatant is substantially free of host cells but contains the cell culture medium and the recombinant virus and hence can be used as a live  
25 vaccine. The supernatant may be diluted by adding a pharmacologically inert carrier, e.g., physiological saline, etc. The supernatant may be freeze-dried to be provided for use as a live vaccine. A method for

administration of the live vaccine of the present invention to fowl is not particularly limited and examples of the administration include a method for scratching the skin and inoculating the live vaccine on the scratch, effecting the inoculation through injection, oral administration by mixing the live vaccine with feed or drinking water, inhalation by aerosol or spray, etc. In order to use as the live vaccine, the dosage may be the same as ordinary live vaccine; for example, approximately  $10^2$  to  $10^8$  plaque forming unit (hereinafter abbreviated as PFU) is inoculated per chick. Where the inoculation is effected by injection, the recombinant virus of the present invention is generally suspended in about 0.1 ml of an isotonic solvent such as physiological saline and the resulting suspension is provided for use. The live vaccine of the present invention can be lyophilized under ordinary conditions and can be stored at room temperature. It is also possible to freeze the virus suspension at -20 to -70°C and store the frozen suspension.

Particularly where the genes coding for the polypeptides derived from the outer membrane proteins of herpes viruses described above are those coding for polypeptides having more than one epitope of herpes viruses, preferably having at least 90% homology to native outer membrane proteins, the live vaccine of the present invention functions as a vaccine for both Mycoplasma gallisepticum infection and Avipox viral

infection. In addition, the live vaccine of the present invention can also function as an effective vaccine for infection with herpes virus originating from outer membrane proteins. That is, the live vaccine of the present invention can be used as a so-called trivalent vaccine.

#### EXAMPLES

##### Example 1

Construction of recombinant pNZ40K-S bearing hybrid DNA ligating TTM-1 protein DNA immediately after the signal of gB gene for Marek's disease virus (cf. Figs. 1, 2 and 3)

First, plasmid pUCgB bearing gB gene of Marek's disease virus, disclosed in JPA 6-78764, was digested with restriction enzymes BamHI and SalI to recover a fragment of 3.9 kb.

Separately, plasmid pGTPs was constructed by digesting plasmid pNZ1729R (Yanagida et al., J. Virol., 66, 1402-1408 (1992)) with HindIII and SalI, inserting the resulting DNA fragment of about 140 bp into pUC18 at the HindIII-SalI site thereof, further inserting synthetic DNA (5'-AGCTGCCCCCGGCAAGCTTGCA-3') at the HindIII-PstI site, then inserting synthetic DNA (5'-TCGACATTTTATGTGTAC-3') at the SalI-EcoRI site and finally inserting synthetic DNA (5'-AATCGCCCGGGGGGCCAGCT-3') at the SacI-EcoRI site.

The thus obtained pGTPs was digested with

restriction enzymes SalI and BamHI and then ligated with the aforesaid 3.9 kb fragment using a ligase to obtain pGTPsMDgB. Thereafter, pNZ2929XM1 disclosed in WO 94/23019 was digested with EcoRI to recover a fragment of 740 bp and then obtained a blunt end with T4 DNA polymerase. On the other hand, pGTPsMDgB was also digested with XbaI and then obtained a blunt end with T4 DNA polymerase. Subsequently, pGTPsMDgB was ligated with the 740 bp fragment having the blunt end using a ligase to construct a new plasmid. This new plasmid was digested with BglII and SalI to recover a fragment of 3.0 kb. The 3.0 kb fragment was ligated with the 1.1 kb fragment obtained through digestion of pNZ2927XM1 with BglII and SalI, using a ligase. Thus, there was obtained a plasmid ligating the N terminus of TTM-1 gene at the C terminus of the signal sequence of gB gene of Marek's disease virus.

Finally, a fragment of 1.4 kb obtained by digestion of pGTPs40K-S with SalI and BamHI was ligated with a fragment of 9.3 kb obtained by digestion of plasmid pNZ1829R with SalII and BamHI, using a ligase. The objective plasmid pNZ40K-S of 10.7 kb was thus constructed for use in recombination.

#### Example 2

25 Construction of recombinant pNZ40K-C bearing hybrid DNA ligating TTM-1 protein DNA at the C terminus of gB gene for Marek's disease virus (cf. Figs. 4, 5 and 6)

After plasmid pGTPsMDgB obtained in Example 1 was digested with restriction enzyme MluI, and then obtained a blunt end with T4 DNA polymerase, which was followed by digestion with restriction enzyme XbaI to

5 recover a fragment of 1.9 kb. Separately, pBluescriptII (made by Toyobo Co., Ltd., hereinafter abbreviated as pBSKSII) was digested with restriction enzymes XbaI and SmaI. The resulting fragment was ligated with the 1.9 kb fragment obtained above using a ligase to give a plasmid.

10 The resulting plasmid was digested with restriction enzymes EcoRI and SalI. The resulting fragment was ligated with the 550 bp fragment and the 615 bp fragment, both obtained by digestion of pNZ2929XM1 with restriction enzymes EcoRI and SalI and with restriction enzymes

15 EcoT22I and SalI, respectively, using a ligase to construct a plasmid. The thus obtained plasmid was digested with restriction enzymes XbaI and SalI. The resulting 2.7 kb fragment was ligated with the 3.3 kb fragment obtained by digestion of pGTPsMDgB with

20 restriction enzymes XbaI and SalI, using a ligase. Plasmid pGTPs40K-C ligating the TTM-1 gene at the N terminus thereof with the gB gene for Marek's disease virus at the C terminus thereof was thus obtained.

Finally, a fragment of 2.7 kb obtained by

25 digestion of pGTPs40K-C with SalI and XbaI was ligated with a fragment of 9.5 kb obtained by digestion of plasmid pNZ1829R with SalI and XbaI, using a ligase. The objective plasmid pNZ40K-C of 12.2 kb for recombination

was thus constructed.

### Example 3

#### Construction of recombinants FPV 40K-C and 40K-S and purification thereof

- 5 NP strain, which is a fowlpox live vaccine strain, was infected to monolayered CEF at m.o.i. = 0.1. Three hours after, these cells were scraped off from the monolayer by a treatment with trypsin to form a cell suspension. After  $2 \times 10^7$  cells in the suspension were
- 10 mixed with 10  $\mu$ g of plasmid pNZ40K-C or pNZ40K-S for use in recombination, the mixture was suspended in Saline G (0.14 M sodium chloride, 0.5 mM potassium chloride, 1.1 mM disodium hydrogenphosphate, 1.5 mM potassium dihydrogenphosphate, 0.5 mM magnesium chloride
- 15 hexahydrate, 0.011% glucose). The suspension was subjected to electrophoresis under conditions of 3.0 kV  $\text{cm}^{-1}$ , 0.4 msec and 25°C, using Gene Pulser (manufactured by Bio-Rad Co., Ltd.) at room temperature. The plasmid-infected cells were then cultured at 37°C for 72 hours.
- 20 The cells were lysed by freezing and thawing 3 times to recover viruses containing the recombinant virus.

- The recovered recombinant virus was selected as follows. The recovered viral solution was infected to monolayered CEF and 10 ml of agar solution containing
- 25 growth medium was overlaid thereon. After agar was warmed at room temperature, incubation was performed at 37°C until plaques of FPV appeared. Then agar medium

containing Blueo-gal in a concentration of 200 µg/ml was overlaid on the agar followed by incubation at 37°C for further 48 hours. Among all of the plaques, about 1% of the plaques were colored blue. These blue plaques were isolated and recovered. By the same procedures, isolation and recovery were repeated to purify the virus until all the plaques were stained to blue. In general, the repeated procedures were terminated by 3 to 4 days. The purified strains were named 40K-C and 40K-S, respectively. In 40K-C and 40K-S, each position of the DNAs inserted was confirmed by dot blotting hybridization and Southern blotting hybridization.

#### Example 4

##### Expression of TTM-1 polypeptide in cells infected with

##### 40K-C and 40K-S

In order to confirm that 40K-C and 40K-S could express TTM-1 polypeptide in infected cells, Western blotting was performed using anti-Mycoplasma gallisepticum S6 strain sera. Virus 40K-C or 40K-S was infected to CEF and cultured at 37°C until plaques were formed. The cells were then scraped off with a cell scraper and centrifuged at 8000G for 20 minutes together with the culture supernatant. The cell-containing precipitates (hereinafter referred to as pellets) were recovered. After washing with PBS, the pellets were centrifuged at 8000G for 20 minutes followed by rinsing to recover the pellets. The pellets were then suspended

- in 150  $\mu$ l of PBS. From the suspension 50  $\mu$ l was taken and added with the same volume of Laemmli's buffer (containing 10% mercapto-ethanol). After boiling for 3 minutes, the mixture was subjected to sodium dodecyl
- 5 sulfate-polyacrylamide gel electrophoresis (hereinafter abbreviated as SDS-PAGE) in accordance with the Laemmli's method (Nature, 227, 668-685 (1970)). The polypeptides isolated on the SDS-PAGE-completed gel were transferred onto a polyvinylidene difluoride membrane (Immobilon
- 10 Transfer Membrane, made by Millipore Inc., hereinafter simply referred to as membrane) according to the method of Burnett et al., (A. Anal. Biochem., 112, 195-203 (1970)) or by the method of Towbin et al. (Proc. Natl. Acad. Sci., 75, 4350-4354 (1979)) by means of
- 15 electrophoresis. The membrane was dipped for an hour into PBS containing 3% skimmed milk for blocking not to cause any non-specific binding. Next, the membrane was dipped for an hour in PBS in which chick anti-Mycoplasma gallisepticum S6 strain serum was diluted to 1000-fold.
- 20 Subsequently, the membrane was rinsed with PBS and then dipped for an hour in PBS containing alkaline phosphatase conjugate anti-chick IgG as a secondary antibody. After the membrane was rinsed with PBS, a color-forming reaction was carried out in 10 ml of a
- 25 solution containing 100 mM Tris hydrochloride (pH 7.5), 0.15 M sodium chloride and 50 mM magnesium chloride, using Nitro Blue Tetrazolium salt (NBT, made by GIBCO-BRL Inc.) and 5-bromo-4-chloro-3-indole phosphate-p-toluidine



(BCIP, made by GIBCO-BRL Inc.) as color-forming substrates.

The results of the Western blotting are shown in Fig. 7.

- 5           As shown in Fig. 7, proteins could be confirmed with the cells infected both with 40K-S and 40K-C as those reactive at the objective positions. It was thus verified that the expected proteins could be expressed in the recombinant FPV infected cells.

10 Example 5

Antibody-inducing capability of recombinant FPV-inoculated chicken

- After 40K-C and 40K-S were cultured in CEF at 37°C for 48 hours, the procedure of freezing and thawing  
15 was repeated twice to recover the cell suspension. The cell suspension was adjusted to have a virus titer of  $10^6$  pfu/ml and then inoculated to SPF chicken (Line M, Nippon Seibutsu Kagaku Kenkyusho) of 7 days old at the right wing web in a dose of 10  $\mu$ l through a stab needle. After  
20 the inoculation, take of the pock was observed and the sera were collected 2 weeks after the inoculation. The antibody titer of the sera collected was determined by ELISA. The purified TTM-1 polypeptide was dissolved in a bicarbonate buffer solution in a concentration of 1  
25  $\mu$ g/well. After adsorption to a 96 well microtiter plate, blocking was effected with skimmed milk to prevent the subsequent non-specific adsorption. Next, a dilution of

the sample serum was charged in each well and then horse  
 radish peroxide-labeled anti-chicken immunoglobulin  
 antibody (rabbit antibody) was added thereto as a  
 secondary antibody. After thoroughly washing, 2,2'-  
 5 azinodiethylbenzothiazoline sulfonate was added to the  
 mixture as a substrate and a relative dilution  
 magnification of the antibody was measured with an  
 immuno-reader in terms of absorbance at a wavelength of  
 405 nm. As a primary antibody for control, anti-TTM-1  
 10 polypeptide chicken serum was used. The results are  
 shown in Table 1.

Table 1. Antibody titer of rFPV-inoculated  
 chicken by ELISA

15	Methods for treating chicken	Antibody titer of anti- TTM-1 polypeptide
	40K-S inoculation	1024
	40K-C inoculation	512
	TTM-1 immunization	512
	non-inoculated	1

20 Antibody titer: Dilution magnification when the  
 antibody titer of the group of non-  
 inoculated chicken serum dilution was  
 made 1

As shown in Table 1, the results reveal that  
 25 when 40K-C or 40K-S was inoculated to chicken, the anti-  
 TTM-1 antibody titer in sera was increased to the level

higher than the antibody titer in sera from the chicken immunized with TTM-1 polypeptide. From the results it was confirmed that the recombinant FPV could significantly induce the antibody titer to the inoculated  
5 chicken.

#### Example 6

##### Mycoplasma challenge test against recombinant FPV-inoculated chicken

The challenge test was conducted basically in  
10 accordance with the standard for biological preparations for animals. The method is briefly described below.

Strains 40K-C and 40K-S were inoculated to SPF chicken (Line M, Japan Biological Science Laboratory) of 5 weeks old at the right wing web in a dose of 10  $\mu$ l  
15 through a stab needle. After the inoculation, take of the pock was observed to verify completion of the immunization. Two weeks after the inoculation, Mycoplasma gallisepticum strain R was forced to be intratracheally administered in a dose of  $10^4$  to  $10^5$   
20 cfu/chick, whereby infection was made sure. On Day 14 after the infection, the chicken were euthanized with Nembutal. Tissue sections were prepared from the tracheal lesion and scores of the tracheal lesion were determined based on the thickness of tracheal mucous  
25 membrane and histological findings. The scores were also determined by the above standard for biological preparations. An average of scores for the tracheal

lesion observed with each chick in the groups was made the score for the respective groups. For information, criteria to determine tracheal lesion scores is shown in Table 2.

Table 2. Standard Criteria for Scoring Tracheal Lesion

Thickness of Mucous Membrane	Histological Finding	Score
90 $\mu$ m ~	normal appearance of ciliated epithelial cells and mucus gland	0
90 $\mu$ m ~ 110 $\mu$ m	In the lamina propria, slight infiltration of round cells or minute nest can be found, but epithelial cell-layer is normal.	1
	Epithelial cell are degenerated or diseminated, and the lamina propria is moderately thickened due to round cells infiltration.	2
110 $\mu$ m ~	Squamous metaplasia of surface epithelium and lamina propria is extremely thickened due to capillary hyperplasia and rounded cells infiltration; cell debris are accumulated in the tracheal lumen.	3

The results of evaluation are shown in Table 3 and Fig. 8.

Table 3. Means tracheal lesion scores in FPV-inoculated Chicken

Vaccination	Lesion Score	
	Average	Standard Error
40K-S	1.38	0.16
40K-C	1.89	0.13
Commercial vaccine	2.11	0.24
TTM-1 polypeptide	1.09	0.23
None	2.27	0.21

As is clearly noted from the results above, the lesion scores of chicken inoculated with 40K-C and 40K-S are obviously low as compared to that of the non-inoculated chicken, indicating that the vaccines of the present invention clearly imparted to chicken the effective infection prevention for Mycoplasma challenge. Thus, the results reveal that 40K-C and 40K-S could be effective vaccines for Mycoplasma gallisepticum.

## INDUSTRIAL APPLICABILITY

According to the present invention, the fusion proteins of the polypeptides derived from antigenic proteins of Mycoplasma gallisepticum and the polypeptides  
5 derived from outer membrane proteins of herpes viruses are obtained. The fusion proteins are effective as vaccines for anti-Mycoplasma infection, anti-chicken pox or anti-Marek's disease. By use of the hybrid DNAs coding for the fusion proteins, Mycoplasma gallisepticum  
10 antigenic proteins can be efficiently provided on the surface of host cells. Moreover, the hybrid DNAs can secrete the antigenic proteins extracellularly to obtain Avipox viruses that can be efficiently recognized by the antigen recognizing cells in host cells. The thus  
15 obtained recombinant Avipox viruses are useful as potent vaccines for anti-Mycoplasma infection.

SEQ NO: 1

Type of sequence: nucleic acid

Topology: linear

Kind of sequence: other nucleic acid, hybrid DNA (40K-S)

Sequence:

ATG CAC TAT TTT AGG CGG AAT TGC ATA TTT TTC CTT ATA GTT ATT CTA 48  
Met His Tyr Phe Arg Arg Asn Cys Ile Phe Phe Leu Ile Val Ile Leu  
1 5 10 15  
TAT GGT ACG AAC TCA TCT CCG AGT ACC CAA AAT GTG ACA TCA AGA GAA 96  
Tyr Gly Thr Asn Ser Ser Pro Ser Thr Gln Asn Val Thr Ser Arg Glu  
20 25 30

	GTT GTT TCG AGC GTC CAG TTG TCT GAG GAA GAG TCT ACG TTT TAT CTT	144
	Val Val Ser Ser Val Gln Leu Ser Glu Glu Glu Ser Thr Phe Tyr Leu	
	35 40 45	
	TGT CCC CCA CCA GTG GGT TCA ACC GTG ATC CGT CTA GAA TTC GGC TGT	192
5	Cys Pro Pro Pro Val Gly Ser Thr Val Ile Arg Leu Glu Phe Gly Cys	
	50 55 60	
	ATG TCT ATT ACT AAA AAA GAT GCA AAC CCA AAT AAT GGC CAA ACC CAA	240
	Met Ser Ile Thr Lys Lys Asp Ala Asn Pro Asn Asn Gly Gln Thr Gln	
	65 70 75 80	
10	TTA GAA GCA GCG CGA ATG GAG TTA ACA GAT CTA ATC AAT GCT AAA GCG	288
	Leu Glu Ala Ala Arg Met Glu Leu Thr Asp Leu Ile Asn Ala Lys Ala	
	85 90 95	
	ATG ACA TTA GCT TCA CTA CAA GAC TAT GCC AAG ATT GAA GCT AGT TTA	336
	Met Thr Leu Ala Ser Leu Gln Asp Tyr Ala Lys Ile Glu Ala Ser Leu	
15	100 105 110	
	TCA TCT GCT TAT AGT GAA GCT GAA ACA GTT AAC AAT AAC CTT AAT GCA	384
	Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val Asn Asn Asn Leu Asn Ala	
	115 120 125	
	ACA TTA GAA CAA CTA AAA ATG GCT AAA ACT AAT TTA GAA TCA GCC ATC	432
20	Thr Leu Glu Gln Leu Lys Met Ala Lys Thr Asn Leu Glu Ser Ala Ile	
	130 135 140	
	AAC CAA GCT AAT ACG GAT AAA ACG ACT TTT GAT AAT GAA CAC CCA AAT	480
	Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe Asp Asn Glu His Pro Asn	
	145 150 155 160	
25	TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC ACT TTA GAA CAA CGT GCT	528
	Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr Thr Leu Glu Gln Arg Ala	
	165 170 175	



	ACT AAC CTT GAA GGT TTG TCA TCA ACT GCT TAT AAT CAA ATT CGC AAT	576
	Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala Tyr Asn Gln Ile Arg Asn	
	180 185 190	
	AAT TTA GTG GAT CTA TAC AAT AAA GCT AGT AGT TTA ATA ACT AAA ACA	624
5	Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser Ser Leu Ile Thr Lys Thr	
	195 200 205	
	CTA GAT CCA CTA AAT GGG GGA ACG CTT TTA GAT TCT AAT GAG ATT ACT	672
	Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu Asp Ser Asn Glu Ile Thr	
	210 215 220	
10	ACA GCT AAT AAG AAT ATT AAT AAT ACG TTA TCA ACT ATT AAT GAA CAA	720
	Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu Ser Thr Ile Asn Glu Gln	
	225 230 235 240	
	AAG ACT AAT GCT GAT GCA TTA TCT AAT AGT TTT ATT AAA AAA GTG ATT	768
	Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser Phe Ile Lys Lys Val Ile	
15	245 250 255	
	CAA AAT AAT GAA CAA AGT TTT GTA GGG ACT TTT ACA AAC CCT AAT GTT	816
	Gln Asn Asn Glu Gln Ser Phe Val Gly Thr Phe Thr Asn Ala Asn Val	
	260 265 270	
	CAA CCT TCA AAC TAC AGT TTT GTT GCT TTT AGT GCT GAT GTA ACA CCC	864
20	Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe Ser Ala Asp Val Thr Pro	
	275 280 285	
	GTC AAT TAT AAA TAT GCA AGA AGG ACC GTT TGG AAT GGT GAT GAA CCT	912
	Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val Trp Asn Gly Asp Glu Pro	
	290 295 300	
25	TCA AGT AGA ATT CTT GCA AAC ACG AAT AGT ATC ACA GAT GTT TCT TGG	960
	Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser Ile Thr Asp Val Ser Trp	
	305 310 315 320	

0017052 010543  
000010 250440

	ATT TAT AGT TTA GCT GGA ACA AAC ACG AAG TAC CAA TTT AGT TTT AGC	1008
	Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys Tyr Gln Phe Ser Phe Ser	
	325 330 335	
	AAC TAT GGT CCA TCA ACT GGT TAT TTA TAT TTC CCT TAT AAG TTG GTT	1056
5	Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr Phe Pro Tyr Lys Leu Val	
	340 345 350	
	AAA GCA GCT GAT GCT AAT AAC GTT GGA TTA CAA TAC AAA TTA AAT AAT	1104
	Lys Ala Ala Asp Ala Asn Asn Val Gly Leu Gln Tyr Lys Leu Asn Asn	
	355 360 365	
10	GGA AAT GTT CAA CAA GTT GAG TTT GCC ACT TCA ACT AGT GCA AAT AAT	1152
	Gly Asn Val Gln Gln Val Glu Phe Ala Thr Ser Thr Ser Ala Asn Asn	
	370 375 380	
	ACT ACA GCT AAT CCA ACT CCA GCA GTT GAT GAG ATT AAA GTT GCT AAA	1200
	Thr Thr Ala Asn Pro Thr Pro Ala Val Asp Glu Ile Lys Val Ala Lys	
15	385 390 395 400	
	ATC GTT TTA TCA GGT TTA AGA TTT GGC CAA AAC ACA ATC GAA TTA AGT	1248
	Ile Val Leu Ser Gly Leu Arg Phe Gly Gln Asn Thr Ile Glu Leu Ser	
	405 410 415	
	GTT CCA ACG GGT GAA GGA AAT ATG AAT AAA GTT GCG CCA ATG ATT GGC	1296
20	Val Pro Thr Gly Glu Gly Asn Met Asn Lys Val Ala Pro Met Ile Gly	
	420 425 430	
	AAC ATT TAT CTT AGC TCA AAT GAA AAT AAT GCT GAT AAG ATC CCC GGG	1344
	Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn Ala Asp Lys Ile Pro Gly	
	435 440 445	
25	TAC CGT CGA CCC GGT ACA TTT TTA TAA	1371
	Tyr Arg Arg Pro Gly Thr Phe Leu ***	
	450 455	

## SEQUENCE LISTING

SEQ NO: 2

Length of sequence: 456

Type of sequence: amino acid

5 Topology: linear

Kind of sequence: protein

Sequence:

Met	His	Tyr	Phe	Arg	Arg	Asn	Cys	Ile	Phe	Phe	Leu	Ile	Val	Ile	Leu
1						5					10				15
Tyr	Gly	Thr	Asn	Ser	Ser	Pro	Ser	Thr	Gln	Asn	Val	Thr	Ser	Arg	Glu
			20						25					30	
Val	Val	Ser	Ser	Val	Gln	Leu	Ser	Glu	Glu	Glu	Ser	Thr	Phe	Tyr	Leu
			35					40					45		
Cys	Pro	Pro	Pro	Val	Gly	Ser	Thr	Val	Ile	Arg	Leu	Glu	Phe	Gly	Cys
			50				55				60				
Met	Ser	Ile	Thr	Lys	Lys	Asp	Ala	Asn	Pro	Asn	Asn	Gly	Gln	Thr	Gln
	65					70				75				80	
Leu	Glu	Ala	Ala	Arg	Met	Glu	Leu	Thr	Asp	Leu	Ile	Asn	Ala	Lys	Ala
				85					90					95	
Met	Thr	Leu	Ala	Ser	Leu	Gln	Asp	Tyr	Ala	Lys	Ile	Glu	Ala	Ser	Leu
			100						105					110	
Ser	Ser	Ala	Tyr	Ser	Glu	Ala	Glu	Thr	Val	Asn	Asn	Asn	Leu	Asn	Ala
			115						120				125		
Thr	Leu	Glu	Gln	Leu	Lys	Met	Ala	Lys	Thr	Asn	Leu	Glu	Ser	Ala	Ile
			130				135					140			
Asn	Gln	Ala	Asn	Thr	Asp	Lys	Thr	Thr	Phe	Asp	Asn	Glu	His	Pro	Asn
			145				150				155				160

Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr Thr Leu Glu Gln Arg Ala			
	165	170	175
Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala Tyr Asn Gln Ile Arg Asn			
	180	185	190
Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser Ser Leu Ile Thr Lys Thr			
	195	200	205
Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu Asp Ser Asn Glu Ile Thr			
	210	215	220
Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu Ser Thr Ile Asn Glu Gln			
225	230	235	240
Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser Phe Ile Lys Lys Val Ile			
	245	250	255
Gln Asn Asn Glu Gln Ser Phe Val Gly Thr Phe Thr Asn Ala Asn Val			
	260	265	270
Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe Ser Ala Asp Val Thr Pro			
	275	280	285
Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val Trp Asn Gly Asp Glu Pro			
	290	295	300
Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser Ile Thr Asp Val Ser Trp			
305	310	315	320
Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys Tyr Gln Phe Ser Phe Ser			
	325	330	335
Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr Phe Pro Tyr Lys Leu Val			
	340	345	350
Lys Ala Ala Asp Ala Asn Asn Val Gly Leu Gln Tyr Lys Leu Asn Asn			
	355	360	365
Gly Asn Val Gln Gln Val Glu Phe Ala Thr Ser Thr Ser Ala Asn Asn			
	370	375	380



SEQ NO: 3

Type of sequence: nucleic acid

5 Number of strand: double strand

Topology: linear

Kind of sequence: other nucleic acid, hybrid DNA (40K-C)

**Sequence:**

ATG CAC TAT TTT AGG CGG AAT TGC ATA TTT TTC CTT ATA GTT ATT CTA 48

Met His Tyr Phe Arg Arg Asn Cys Ile Phe Phe Leu Ile Val Ile Leu

1                      5                      10                      15

TAT GGT ACG AAC TCA TCT CCG AGT ACC CAA AAT GTG ACA TCA AGA GAA 96

Tyr Gly Thr Asn Ser Ser Pro Ser Thr Gln Asn Val Thr Ser Arg Glu

20                      25                      30

GTT GTT TCG AGC GTC CAG TTG TCT GAG GAA GAG TCT ACG TTT TAT CTT 144

Val Val Ser Ser Val Gln Leu Ser Glu Glu Glu Ser Thr Phe Tyr Leu

35                      40                      45

TGT CCC CCA CCA GTG GGT TCA ACC GTG ATC CGT CTA GAA CCG CCG CGA	192
Cys Pro Pro Pro Val Gly Ser Thr Val Ile Arg Leu Glu Pro Pro Arg	
50 55 60	
AAA TGT CCC GAA CCT AGA AAA GCC ACC GAG TGG GGT GAA GGA ATC GCG	240
Lys Cys Pro Glu Pro Arg Lys Ala Thr Glu Trp Gly Glu Gly Ile Ala	
65 70 75 80	
ATA TTA TTT AAA GAG AAT ATC AGT CCA TAT AAA TTT AAA GTG ACG CTT	288
Ile Leu Phe Lys Glu Asn Ile Ser Pro Tyr Lys Phe Lys Val Thr Leu	
85 90 95	
TAT TAT AAA AAT ATC ATT CAG ACG ACG ACA TGG ACG GGG ACG ACA TAT	336
Tyr Tyr Lys Asn Ile Ile Gln Thr Thr Thr Trp Thr Gly Thr Thr Tyr	
100 105 110	
AGA CAG ATC ACT AAT CGA TAT ACA GAT AGG ACG CCC GTT TCC ATT GAA	384
Arg Gln Ile Thr Asn Arg Tyr Thr Asp Arg Thr Pro Val Ser Ile Glu	
115 120 125	
GAG ATC ACG GAT CTA ATC GAC GGC AAA GGA AGA TGC TCA TCT AAA GCA	432
Glu Ile Thr Asp Leu Ile Asp Gly Lys Gly Arg Cys Ser Ser Lys Ala	
130 135 140	
AGA TAC CTT AGA AAC AAT GTA TAT GTT GAA GCG TTT GAC AGG GAT GCG	480
Arg Tyr Leu Arg Asn Asn Val Tyr Val Glu Ala Phe Asp Arg Asp Ala	
145 150 155 160	
GGA GAA AAA CAA GTA CTT CTA AAA CCA TCA AAA TTC AAC ACG CCC GAA	528
Gly Glu Lys Gln Val Leu Leu Lys Pro Ser Lys Phe Asn Thr Pro Glu	
165 170 175	
TCT AGG GCA TGG CAC ACG ACT AAT GAG ACG TAT ACC GTG TGG GGA TCA	576
Ser Arg Ala Trp His Thr Thr Asn Glu Thr Tyr Thr Val Trp Gly Ser	
180 185 190	

CCA TGG ATA TAT CGA ACG GGA ACC TCC GTC AAT TGT ATA GTA GAG GAA	624
Pro Trp Ile Tyr Arg Thr Gly Thr Ser Val Asn Cys Ile Val Glu Glu	
195 200 205	
ATG GAT GCC CGC TCT GTG TTT CCG TAT TCA TAT TTT GCA ATG GCC AAT	672
Met Asp Ala Arg Ser Val Phe Pro Tyr Ser Tyr Phe Ala Met Ala Asn	
210 215 220	
GGC GAC ATC GCG AAC ATA TCT CCA TTT TAT GGT CTA TCC CCA CCA GAG	720
Gly Asp Ile Ala Asn Ile Ser Pro Phe Tyr Gly Leu Ser Pro Pro Glu	
225 230 235 240	
GCT GCC GCA GAA CCC ATG GGA TAT CCC CAG GAT AAT TTC AAA CAA CTA	768
Ala Ala Ala Glu Pro Met Gly Tyr Pro Gln Asp Asn Phe Lys Gln Leu	
245 250 255	
GAT AGC TAT TTT TCA ATG GAT TTG GAC AAG CGT CGA AAA GCA AGC CTT	816
Asp Ser Tyr Phe Ser Met Asp Leu Asp Lys Arg Arg Lys Ala Ser Leu	
260 265 270	
CCA GTC AAG CGT AAC TTT CTC ATC ACA TCA CAC TTC ACA GTT GGG TGG	864
Pro Val Lys Arg Asn Phe Leu Ile Thr Ser His Phe Thr Val Gly Trp	
275 280 285	
GAC TGG GCT CCA AAA ACT ACT CGT GTA TGT TCA ATG ACT AAG TGG AAA	912
Asp Trp Ala Pro Lys Thr Thr Arg Val Cys Ser Met Thr Lys Trp Lys	
290 295 300	
GAG GTG ACT GAA ATG TTG CGT GCA ACA GTT AAT GGG AGA TAC AGA TTT	960
Glu Val Thr Glu Met Leu Arg Ala Thr Val Asn Gly Arg Tyr Arg Phe	
305 310 315 320	
ATG GCC CGT GAA CTT TCG GCA ACG TTT ATC AGT AAT ACG ACT GAG TTT	1008
Met Ala Arg Glu Leu Ser Ala Thr Phe Ile Ser Asn Thr Thr Glu Phe	
325 330 335	



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340 345 350	
GCA GCA ATC GAG CAG ATA TTT AGG ACA AAA TAT AAT GAC AGT CAC GTC	1104
Ala Ala Ile Glu Gln Ile Phe Arg Thr Lys Tyr Asn Asp Ser His Val	
355 360 365	
AAG GTT GGA CAT GTA CAA TAT TTC TTG GCT CTC GGG GGA TTT ATT GTA	1152
Lys Val Gly His Val Gln Tyr Phe Leu Ala Leu Gly Gly Phe Ile Val	
370 375 380	
GCA TAT CAG CCT GTT CTA TCC AAA TCC CTG GCT CAT ATG TAC CTC AGA	1200
Ala Tyr Gln Pro Val Leu Ser Lys Ser Leu Ala His Met Tyr Leu Arg	
385 390 395 400	
GAA TTG ATG AGA GAC AAC AGG ACC GAT GAG ATG CTC GAC CTG GTA AAC	1248
Glu Leu Met Arg Asp Asn Arg Thr Asp Glu Met Leu Asp Leu Val Asn	
405 410 415	
AAT AAG CAT GCA ATT TAT AAG AAA AAT GCT ACC TCA TTG TCA CGA TTG	1296
Asn Lys His Ala Ile Tyr Lys Lys Asn Ala Thr Ser Leu Ser Arg Leu	
420 425 430	
CGG CGA GAT ATT CGA AAT GCA CCA AAT AGA AAA ATA ACA TTA GAC GAC	1344
Arg Arg Asp Ile Arg Asn Ala Pro Asn Arg Lys Ile Thr Leu Asp Asp	
435 440 445	
ACC ACA GCT ATT AAA TCG ACA TCG TCT GTT CAA TTC GCC ATG CTC CAA	1392
Thr Thr Ala Ile Lys Ser Thr Ser Ser Val Gln Phe Ala Met Leu Gln	
450 455 460	
TTT CTT TAT GAT CAT ATA CAA ACC CAT ATT AAT GAT ATG TTT AGT AGG	1440
Phe Leu Tyr Asp His Ile Gln Thr His Ile Asn Asp Met Phe Ser Arg	
465 470 475 480	

ATT GCC ACA GCT TGG TGC GAA TTG CAG AAT AGA GAA CTT GTT TTA TGG	1488
Ile Ala Thr Ala Trp Cys Glu Leu Gln Asn Arg Glu Leu Val Leu Trp	
485 490 495	
CAC GAA GGG ATA AAG ATT AAT CCT AGC GCT ACA GCG AGT GCA ACA TTA	1536
His Glu Gly Ile Lys Ile Asn Pro Ser Ala Thr Ala Ser Ala Thr Leu	
500 505 510	
GGA AGG AGA GTG GCT GCA AAG ATG TTG GGG GAT GTC GCT GCT GTA TCG	1584
Gly Arg Arg Val Ala Ala Lys Met Leu Gly Asp Val Ala Ala Val Ser	
515 520 525	
AGC TGC ACT GCT ATA GAT GCG GAA TCC GTC ACT TTG CAA AAT TCT ATG	1632
Ser Cys Thr Ala Ile Asp Ala Glu Ser Val Thr Leu Gln Asn Ser Met	
530 535 540	
CGA GTT ATC ACA TCC ACT AAT ACA TGT TAT AGC CGA CCA TTG GTT CTA	1680
Arg Val Ile Thr Ser Thr Asn Thr Cys Tyr Ser Arg Pro Leu Val Leu	
545 550 555 560	
TTT TCA TAT GGA GAA AAC CAA GGA AAC ATA CAG GGA CAA CTC GGT GAA	1728
Phe Ser Tyr Gly Glu Asn Gln Gly Asn Ile Gln Gly Gln Leu Gly Glu	
565 570 575	
AAC AAC GAG TTG CTT CCA ACG CTA GAG GCT GTA GAG CCA TGC TCG GCT	1776
Asn Asn Glu Leu Leu Pro Thr Leu Glu Ala Val Glu Pro Cys Ser Ala	
580 585 590	
AAT CAT CGT AGA TAT TTT CTG TTT GGA TCC GGT TAT GCT TTA TTT GAA	1824
Asn His Arg Arg Tyr Phe Leu Phe Gly Ser Gly Tyr Ala Leu Phe Glu	
595 600 605	
AAC TAT AAT TTT GTT AAG ATG GTA GAC GCT GCC GAT ATA CAG ATT GCT	1872
Asn Tyr Asn Phe Val Lys Met Val Asp Ala Ala Asp Ile Gln Ile Ala	
610 615 620	

AGC	ACA	TTT	GTC	GAG	CTT	AAT	CTA	ACC	CTG	CTA	GAA	GAT	CGG	GAA	ATT	1920
Ser	Thr	Phe	Val	Glu	Leu	Asn	Leu	Thr	Leu	Leu	Glu	Asp	Arg	Glu	Ile	
625				630					635					640		
TTG	CCT	TTA	TCC	GTT	TAC	ACA	AAA	GAA	GAG	TTG	CGT	GAT	GTT	GGT	GTA	1968
Leu	Pro	Leu	Ser	Val	Tyr	Thr	Lys	Glu	Glu	Leu	Arg	Asp	Val	Gly	Val	
				645					650					655		
TTG	GAT	TAT	GCA	GAA	GTA	GCT	CGC	CGC	AAT	CAA	CTA	CAT	GAA	CTT	AAA	2016
Leu	Asp	Tyr	Ala	Glu	Val	Ala	Arg	Arg	Asn	Gln	Leu	His	Glu	Leu	Lys	
				660					665					670		
TTT	TAT	GAC	ATA	AAC	AAA	GTA	ATA	GAA	GTG	GAT	ACA	AAT	TAC	GCG	GGG	2064
Phe	Tyr	Asp	Ile	Asn	Lys	Val	Ile	Glu	Val	Asp	Thr	Asn	Tyr	Ala	Gly	
				675					680					685		
CTG	CAG	GAA	TTC	GGC	TGT	ATG	TCT	ATT	ACT	AAA	AAA	GAT	GCA	AAC	CCA	2112
Leu	Gln	Glu	Phe	Gly	Cys	Met	Ser	Ile	Thr	Lys	Lys	Asp	Ala	Asn	Pro	
				690					695					700		
AAT	AAT	GGC	CAA	ACC	CAA	TTA	GAA	GCA	GCG	CGA	ATG	GAG	TTA	ACA	GAT	2160
Asn	Asn	Gly	Gln	Thr	Gln	Leu	Glu	Ala	Ala	Arg	Met	Glu	Leu	Thr	Asp	
705				710					715					720		
CTA	ATC	AAT	GCT	AAA	GCG	ATG	ACA	TTA	GCT	TCA	CTA	CAA	GAC	TAT	GCC	2208
Leu	Ile	Asn	Ala	Lys	Ala	Met	Thr	Leu	Ala	Ser	Leu	Gln	Asp	Tyr	Ala	
				725					730					735		
AAG	ATT	GAA	GCT	AGT	TTA	TCA	TCT	GCT	TAT	AGT	GAA	GCT	GAA	ACA	GTT	2256
Lys	Ile	Glu	Ala	Ser	Leu	Ser	Ser	Ala	Tyr	Ser	Glu	Ala	Glu	Thr	Val	
				740					745					750		
AAC	AAT	AAC	CTT	AAT	GCA	ACA	TTA	GAA	CAA	CTA	AAA	ATG	GCT	AAA	ACT	2304
Asn	Asn	Asn	Leu	Asn	Ala	Thr	Leu	Glu	Gln	Leu	Lys	Met	Ala	Lys	Thr	
				755					760					765		

AAT TTA GAA TCA GCC ATC AAC CAA GCT AAT ACG GAT AAA ACG ACT TTT	2352
Asn Leu Glu Ser Ala Ile Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe	
770 775 780	
GAT AAT GAA CAC CCA AAT TTA GTT GAA GCA TAC AAA GCA CTA AAA ACC	2400
Asp Asn Glu His Pro Asn Leu Val Glu Ala Tyr Lys Ala Leu Lys Thr	
785 790 795 800	
ACT TTA GAA CAA CGT GCT ACT AAC CTT GAA GGT TTG TCA TCA ACT GCT	2448
Thr Leu Glu Gln Arg Ala Thr Asn Leu Glu Gly Leu Ser Ser Thr Ala	
805 810 815	
TAT AAT CAA ATT CGC AAT AAT TTA GTG GAT CTA TAC AAT AAA GCT AGT	2496
Tyr Asn Gln Ile Arg Asn Asn Leu Val Asp Leu Tyr Asn Lys Ala Ser	
820 825 830	
AGT TTA ATA ACT AAA ACA CTA GAT CCA CTA AAT GGG GGA ACG CTT TTA	2544
Ser Leu Ile Thr Lys Thr Leu Asp Pro Leu Asn Gly Gly Thr Leu Leu	
835 840 845	
GAT TCT AAT GAG ATT ACT ACA GCT AAT AAG AAT ATT AAT AAT ACG TTA	2592
Asp Ser Asn Glu Ile Thr Thr Ala Asn Lys Asn Ile Asn Asn Thr Leu	
850 855 860	
TCA ACT ATT AAT GAA CAA AAG ACT AAT GCT GAT GCA TTA TCT AAT AGT	2640
Ser Thr Ile Asn Glu Gln Lys Thr Asn Ala Asp Ala Leu Ser Asn Ser	
865 870 875 880	
TTT ATT AAA AAA GTG ATT CAA AAT AAT GAA CAA AGT TTT GTA GGG ACT	2688
Phe Ile Lys Lys Val Ile Gln Asn Asn Glu Gln Ser Phe Val Gly Thr	
885 890 895	
TTT ACA AAC GCT AAT GTT CAA CCT TCA AAC TAC AGT TTT GTT GCT TTT	2736
Phe Thr Asn Ala Asn Val Gln Pro Ser Asn Tyr Ser Phe Val Ala Phe	
900 905 910	

AGT GCT GAT GTA ACA CCC GTC AAT TAT AAA TAT GCA AGA AGG ACC GTT	2784
Ser Ala Asp Val Thr Pro Val Asn Tyr Lys Tyr Ala Arg Arg Thr Val	
915 920 925	
TGG AAT GGT GAT GAA CCT TCA AGT AGA ATT CTT GCA AAC ACG AAT AGT	2832
Trp Asn Gly Asp Glu Pro Ser Ser Arg Ile Leu Ala Asn Thr Asn Ser	
930 935 940	
ATC ACA GAT GTT TCT TGG ATT TAT AGT TTA GCT GGA ACA AAC ACG AAG	2880
Ile Thr Asp Val Ser Trp Ile Tyr Ser Leu Ala Gly Thr Asn Thr Lys	
945 950 955 960	
TAC CAA TTT AGT TTT AGC AAC TAT GGT CCA TCA ACT GGT TAT TTA TAT	2928
Tyr Gln Phe Ser Phe Ser Asn Tyr Gly Pro Ser Thr Gly Tyr Leu Tyr	
965 970 975	
TTC CCT TAT AAG TTG GTT AAA GCA GCT GAT GCT AAT AAC GTT GGA TTA	2976
Phe Pro Tyr Lys Leu Val Lys Ala Ala Asp Ala Asn Asn Val Gly Leu	
980 985 990	
CAA TAC AAA TTA AAT AAT GGA AAT GTT CAA CAA GTT GAG TTT GCC ACT	3024
Gln Tyr Lys Leu Asn Asn Gly Asn Val Gln Gln Val Glu Phe Ala Thr	
995 1000 1005	
TCA ACT AGT GCA AAT AAT ACT ACA GCT AAT CCA ACT CCA GCA GTT GAT	3072
Ser Thr Ser Ala Asn Asn Thr Thr Ala Asn Pro Thr Pro Ala Val Asp	
1010 1015 1020	
GAG ATT AAA GTT GCT AAA ATC GTT TTA TCA GGT TTA AGA TTT GGC CAA	3120
Glu Ile Lys Val Ala Lys Ile Val Leu Ser Gly Leu Arg Phe Gly Gln	
1025 1030 1035 1040	
AAC ACA ATC GAA TTA AGT GTT CCA ACG GGT GAA GCA AAT ATG AAT AAA	3168
Asn Thr Ile Glu Leu Ser Val Pro Thr Gly Glu Gly Asn Met Asn Lys	
1045 1050 1055	

GTT GCG CCA ATG ATT GGC AAC ATT TAT CTT AGC TCA AAT GAA AAT AAT 3216

Val Ala Pro Met Ile Gly Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn

1060

1065

1070

GCT GAT AAG ATC CCC GGG TAC CGT CGA CCC GGT ACA TTT TTA TAA 3261

Ala Asp Lys Ile Pro Gly Tyr Arg Arg Pro Gly Thr Phe Leu \*\*\*

1075

1080

1085

## SEQUENCE LISTING

SEQ NO: 4

Length of sequence: 1086

Type of sequence: amino acid

5 Topology: linear

Kind of sequence: protein

Sequence:

Met	His	Tyr	Phe	Arg	Arg	Asn	Cys	Ile	Phe	Phe	Leu	Ile	Val	Ile	Leu
1						5					10				15
Tyr	Gly	Thr	Asn	Ser	Ser	Pro	Ser	Thr	Gln	Asn	Val	Thr	Ser	Arg	Glu
			20						25					30	
Val	Val	Ser	Ser	Val	Gln	Leu	Ser	Glu	Glu	Glu	Ser	Thr	Phe	Tyr	Leu
			35						40					45	
Cys	Pro	Pro	Pro	Val	Gly	Ser	Thr	Val	Ile	Arg	Leu	Glu	Pro	Pro	Arg
			50						55					60	
Lys	Cys	Pro	Glu	Pro	Arg	Lys	Ala	Thr	Glu	Trp	Gly	Glu	Gly	Ile	Ala
			65						70					75	
Ile	Leu	Phe	Lys	Glu	Asn	Ile	Ser	Pro	Tyr	Lys	Phe	Lys	Val	Thr	Leu
					85					90				95	
Tyr	Tyr	Lys	Asn	Ile	Ile	Gln	Thr	Thr	Thr	Trp	Thr	Gly	Thr	Thr	Tyr
					100					105					110

Arg	Gln	Ile	Thr	Asn	Arg	Tyr	Thr	Asp	Arg	Thr	Pro	Val	Ser	Ile	Glu
115				120				125							
Glu	Ile	Thr	Asp	Leu	Ile	Asp	Gly	Lys	Gly	Arg	Cys	Ser	Ser	Lys	Ala
130				135				140							
Arg	Tyr	Leu	Arg	Asn	Asn	Val	Tyr	Val	Glu	Ala	Phe	Asp	Arg	Asp	Ala
145				150				155				160			
Gly	Glu	Lys	Gln	Val	Leu	Leu	Lys	Pro	Ser	Lys	Phe	Asn	Thr	Pro	Glu
165				170				175							
Ser	Arg	Ala	Trp	His	Thr	Thr	Asn	Glu	Thr	Tyr	Thr	Val	Trp	Gly	Ser
180				185				190							
Pro	Trp	Ile	Tyr	Arg	Thr	Gly	Thr	Ser	Val	Asn	Cys	Ile	Val	Glu	Glu
195				200				205							
Met	Asp	Ala	Arg	Ser	Val	Phe	Pro	Tyr	Ser	Tyr	Phe	Ala	Met	Ala	Asn
210				215				220							
Gly	Asp	Ile	Ala	Asn	Ile	Ser	Pro	Phe	Tyr	Gly	Leu	Ser	Pro	Pro	Glu
225				230				235				240			
Ala	Ala	Ala	Glu	Pro	Met	Gly	Tyr	Pro	Gln	Asp	Asn	Phe	Lys	Gln	Leu
245				250				255							
Asp	Ser	Tyr	Phe	Ser	Met	Asp	Leu	Asp	Lys	Arg	Arg	Lys	Ala	Ser	Leu
260				265				270							
Pro	Val	Lys	Arg	Asn	Phe	Leu	Ile	Thr	Ser	His	Phe	Thr	Val	Gly	Trp
275				280				285							
Asp	Trp	Ala	Pro	Lys	Thr	Thr	Arg	Val	Cys	Ser	Met	Thr	Lys	Trp	Lys
290				295				300							
Glu	Val	Thr	Glu	Met	Leu	Arg	Ala	Thr	Val	Asn	Gly	Arg	Tyr	Arg	Phe
305				310				315				320			
Met	Ala	Arg	Glu	Leu	Ser	Ala	Thr	Phe	Ile	Ser	Asn	Thr	Thr	Glu	Phe
325				330				335							



Asp Pro Asn Arg Ile Ile Leu Gly Gln Cys Ile Lys Arg Glu Ala Glu  
 340 345 350  
 Ala Ala Ile Glu Gln Ile Phe Arg Thr Lys Tyr Asn Asp Ser His Val  
 355 360 365  
 Lys Val Gly His Val Gln Tyr Phe Leu Ala Leu Gly Gly Phe Ile Val  
 370 375 380  
 Ala Tyr Gln Pro Val Leu Ser Lys Ser Leu Ala His Met Tyr Leu Arg  
 385 390 395 400  
 Glu Leu Met Arg Asp Asn Arg Thr Asp Glu Met Leu Asp Leu Val Asn  
 405 410 415  
 Asn Lys His Ala Ile Tyr Lys Lys Asn Ala Thr Ser Leu Ser Arg Leu  
 420 425 430  
 Arg Arg Asp Ile Arg Asn Ala Pro Asn Arg Lys Ile Thr Leu Asp Asp  
 435 440 445  
 Thr Thr Ala Ile Lys Ser Thr Ser Ser Val Gln Phe Ala Met Leu Gln  
 450 455 460  
 Phe Leu Tyr Asp His Ile Gln Thr His Ile Asn Asp Met Phe Ser Arg  
 465 470 475 480  
 Ile Ala Thr Ala Trp Cys Glu Leu Gln Asn Arg Glu Leu Val Leu Trp  
 485 490 495  
 His Glu Gly Ile Lys Ile Asn Pro Ser Ala Thr Ala Ser Ala Thr Leu  
 500 505 510  
 Gly Arg Arg Val Ala Ala Lys Met Leu Gly Asp Val Ala Ala Val Ser  
 515 520 525  
 Ser Cys Thr Ala Ile Asp Ala Glu Ser Val Thr Leu Gln Asn Ser Met  
 530 535 540  
 Arg Val Ile Thr Ser Thr Asn Thr Cys Tyr Ser Arg Pro Leu Val Leu  
 545 550 555 560

Phe Ser Tyr Gly Glu Asn Gln Gly Asn Ile Gln Gly Gln Leu Gly Glu  
 565 570 575  
 Asn Asn Glu Leu Leu Pro Thr Leu Glu Ala Val Glu Pro Cys Ser Ala  
 580 585 590  
 Asn His Arg Arg Tyr Phe Leu Phe Gly Ser Gly Tyr Ala Leu Phe Glu  
 595 600 605  
 Asn Tyr Asn Phe Val Lys Met Val Asp Ala Ala Asp Ile Gln Ile Ala  
 610 615 620  
 Ser Thr Phe Val Glu Leu Asn Leu Thr Leu Leu Glu Asp Arg Glu Ile  
 625 630 635 640  
 Leu Pro Leu Ser Val Tyr Thr Lys Glu Glu Leu Arg Asp Val Gly Val  
 645 650 655  
 Leu Asp Tyr Ala Glu Val Ala Arg Arg Asn Gln Leu His Glu Leu Lys  
 660 665 670  
 Phe Tyr Asp Ile Asn Lys Val Ile Glu Val Asp Thr Asn Tyr Ala Gly  
 675 680 685  
 Leu Gln Glu Phe Gly Cys Met Ser Ile Thr Lys Lys Asp Ala Asn Pro  
 690 695 700  
 Asn Asn Gly Gln Thr Gln Leu Glu Ala Ala Arg Met Glu Leu Thr Asp  
 705 710 715 720  
 Leu Ile Asn Ala Lys Ala Met Thr Leu Ala Ser Leu Gln Asp Tyr Ala  
 725 730 735  
 Lys Ile Glu Ala Ser Leu Ser Ser Ala Tyr Ser Glu Ala Glu Thr Val  
 740 745 750  
 Asn Asn Asn Leu Asn Ala Thr Leu Glu Gln Leu Lys Met Ala Lys Thr  
 755 760 765  
 Asn Leu Glu Ser Ala Ile Asn Gln Ala Asn Thr Asp Lys Thr Thr Phe  
 770 775 780

Asp	Asn	Glu	His	Pro	Asn	Leu	Val	Glu	Ala	Tyr	Lys	Ala	Leu	Lys	Thr
785					790					795				800	
Thr	Leu	Glu	Gln	Arg	Ala	Thr	Asn	Leu	Glu	Gly	Leu	Ser	Ser	Thr	Ala
				805						810				815	
Tyr	Asn	Gln	Ile	Arg	Asn	Asn	Leu	Val	Asp	Leu	Tyr	Asn	Lys	Ala	Ser
			820						825					830	
Ser	Leu	Ile	Thr	Lys	Thr	Leu	Asp	Pro	Leu	Asn	Gly	Gly	Thr	Leu	Leu
		835						840						845	
Asp	Ser	Asn	Glu	Ile	Thr	Thr	Ala	Asn	Lys	Asn	Ile	Asn	Asn	Thr	Leu
	850						855							860	
Ser	Thr	Ile	Asn	Glu	Gln	Lys	Thr	Asn	Ala	Asp	Ala	Leu	Ser	Asn	Ser
865				870						875				880	
Phe	Ile	Lys	Lys	Val	Ile	Gln	Asn	Asn	Glu	Gln	Ser	Phe	Val	Gly	Thr
			885							890				895	
Phe	Thr	Asn	Ala	Asn	Val	Gln	Pro	Ser	Asn	Tyr	Ser	Phe	Val	Ala	Phe
		900								905				910	
Ser	Ala	Asp	Val	Thr	Pro	Val	Asn	Tyr	Lys	Tyr	Ala	Arg	Arg	Thr	Val
		915							920					925	
Trp	Asn	Gly	Asp	Glu	Pro	Ser	Ser	Arg	Ile	Leu	Ala	Asn	Thr	Asn	Ser
	930								935					940	
Ile	Thr	Asp	Val	Ser	Trp	Ile	Tyr	Ser	Leu	Ala	Gly	Thr	Asn	Thr	Lys
945				950						955				960	
Tyr	Gln	Phe	Ser	Phe	Ser	Asn	Tyr	Gly	Pro	Ser	Thr	Gly	Tyr	Leu	Tyr
				965						970				975	
Phe	Pro	Tyr	Lys	Leu	Val	Lys	Ala	Ala	Asp	Ala	Asn	Asn	Val	Gly	Leu
			980							985				990	
Gln	Tyr	Lys	Leu	Asn	Asn	Gly	Asn	Val	Gln	Gln	Val	Glu	Phe	Ala	Thr
	995								1000					1005	

Ser Thr Ser Ala Asn Asn Thr Thr Ala Asn Pro Thr Pro Ala Val Asp  
 1010 1015 1020  
 Glu Ile Lys Val Ala Lys Ile Val Leu Ser Gly Leu Arg Phe Gly Gln  
 1025 1030 1035 1040  
 Asn Thr Ile Glu Leu Ser Val Pro Thr Gly Glu Gly Asn Met Asn Lys  
 1045 1050 1055  
 Val Ala Pro Met Ile Gly Asn Ile Tyr Leu Ser Ser Asn Glu Asn Asn  
 1060 1065 1070  
 Ala Asp Lys Ile Pro Gly Tyr Arg Arg Pro Gly Thr Phe Leu \*\*\*  
 1075 1080 1085

## CLAIMS

1. A fusion protein comprising a polypeptide having the antigenicity of Mycoplasma gallisepticum and a polypeptide derived from Herpesvirus outer membrane protein, said polypeptide derived from the outer membrane protein being ligated with the polypeptide having the antigenicity of Mycoplasma gallisepticum at the N terminus thereof.
2. A fusion protein according to claim 1, wherein  
10 said outer membrane protein is derived from a herpes virus showing infection to fowl.
3. A fusion protein according to claim 2, wherein said outer membrane protein is derived from a Marek's disease virus.
- 15 4. A fusion protein according to claim 3, wherein said outer membrane protein is gB protein derived from a Marek's disease virus.
5. A fusion protein according to claim 1, wherein said polypeptide derived from the outer membrane protein  
20 is a signal sequence portion in the outer membrane protein derived from a herpes virus.
6. A fusion protein according to claim 5, wherein said outer membrane protein is a signal sequence portion in the outer membrane protein derived from a herpes virus  
25 showing infection to fowl.
7. A fusion protein according to claim 5, wherein said signal sequence portion is a signal sequence portion in derived from the outer membrane protein of a Marek's

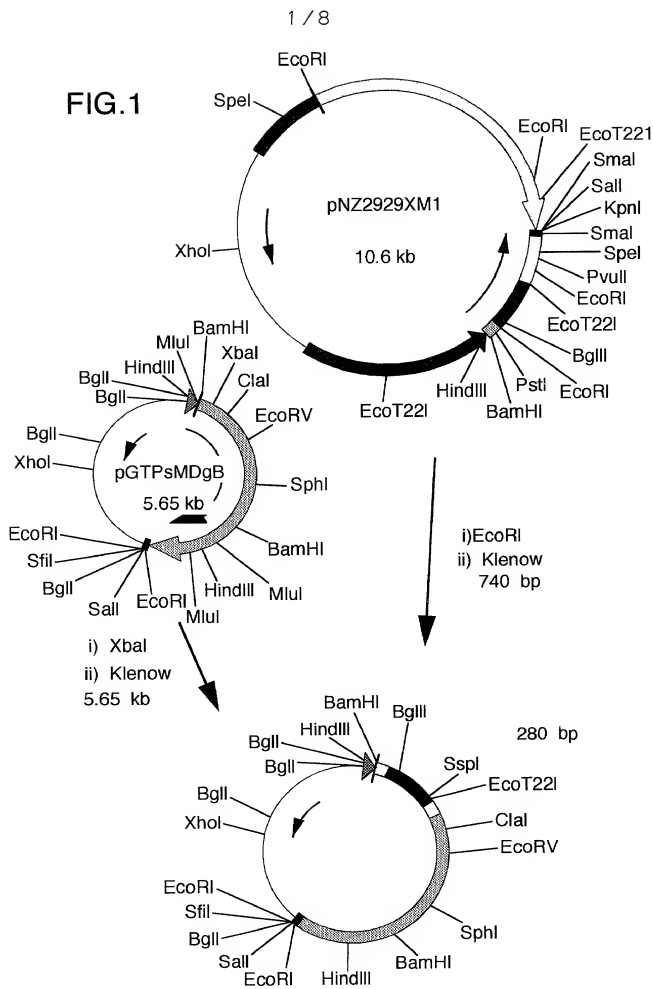
disease virus.

8. A fusion protein according to claim 5, wherein said polypeptide derived from the outer membrane protein is a signal sequence portion of gB protein derived from a
- 5 Marek's disease virus.
9. A hybrid DNA coding for the fusion protein according to any one of claims 1 through 8.
10. A recombinant vector in which a DNA coding for the fusion protein according to any one of claims 1
- 10 through 8 has been inserted.
11. A recombinant Avipox virus in which a DNA coding for the fusion protein according to any one of claims 1 through 8 has been inserted.
12. A recombinant live vaccine for anti-fowl
- 15 Mycoplasma gallisepticum infection comprising as an effective ingredient a recombinant Avipox virus in which a DNA coding for the fusion protein according to any one of claims 1 through 8 has been inserted.
13. A trivalent live vaccine for anti-fowl
- 20 Mycoplasma gallisepticum infection and anti-Marek's disease infection comprising as an effective ingredient a DNA coding for the fusion protein according to any one of claims 3 and 4.

## ABSTRACT

A DNA coding for a fusion protein comprising a polypeptide having the antigenicity of Mycoplasma gallisepticum and a polypeptide derived from Herpesvirus  
5 outer membrane protein, in which the polypeptide derived from the outer membrane protein has been ligated with the polypeptide having the antigenicity of Mycoplasma gallisepticum at the N terminus thereof, is prepared. The DNA is inserted into a region non-essential to growth  
10 of Avipox virus and the resulting recombinant Avipox virus is provided as a more potent recombinant virus as an anti-Mycoplasma vaccine.

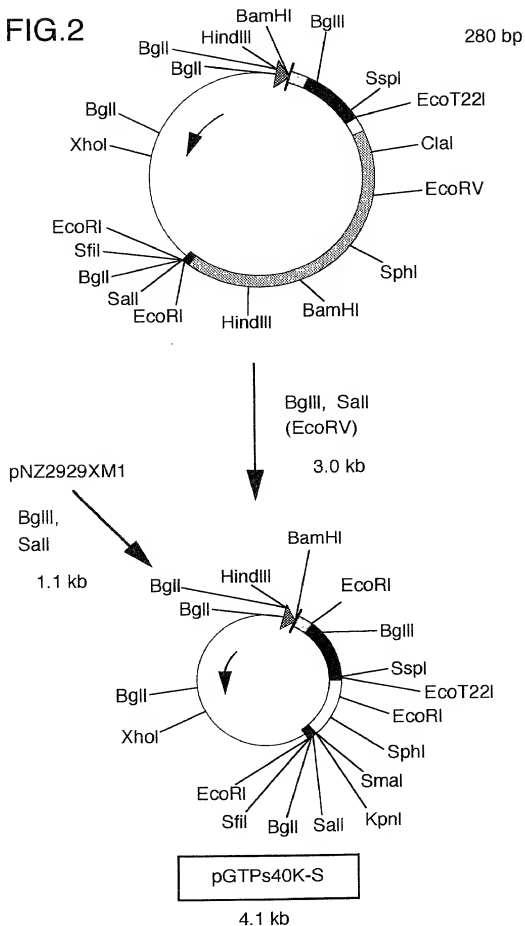
FIG.1





2 / 8

FIG.2



3 / 8

FIG.3

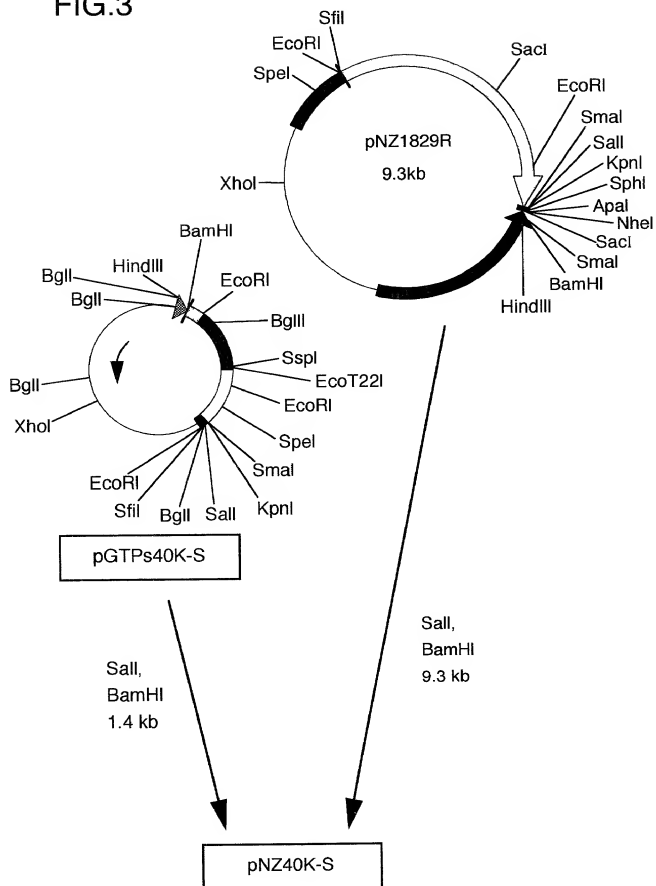
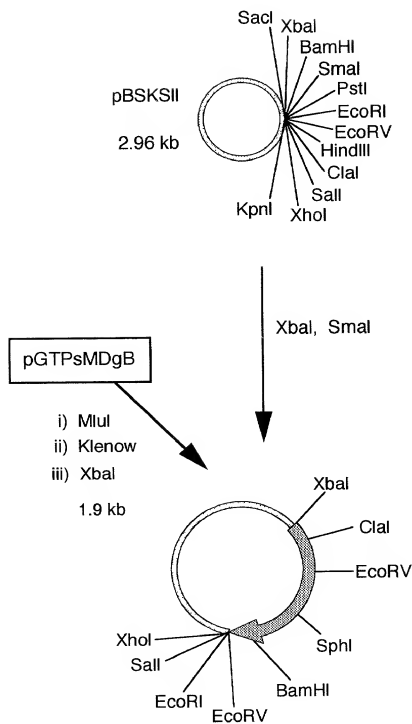
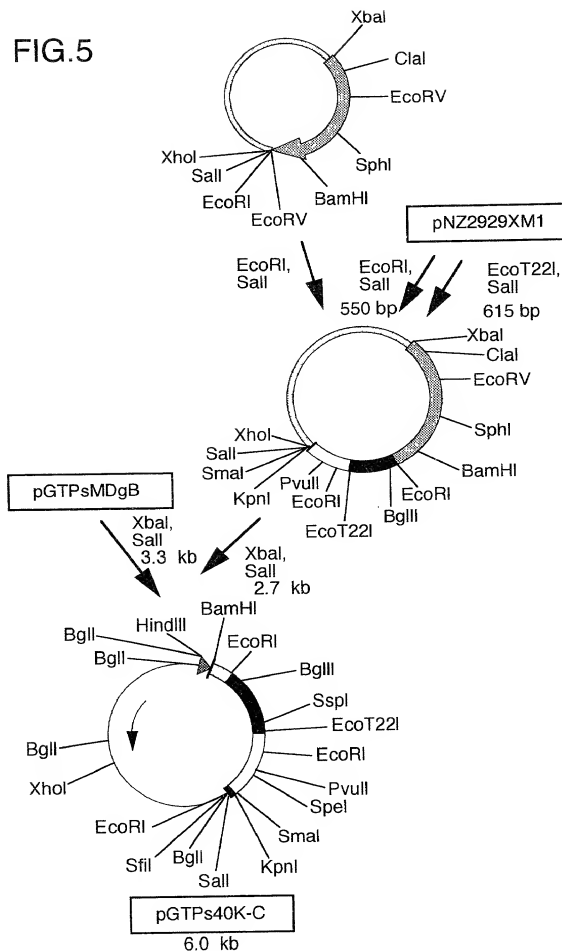


FIG.4



5 / 8

FIG.5



6 / 8

FIG.6

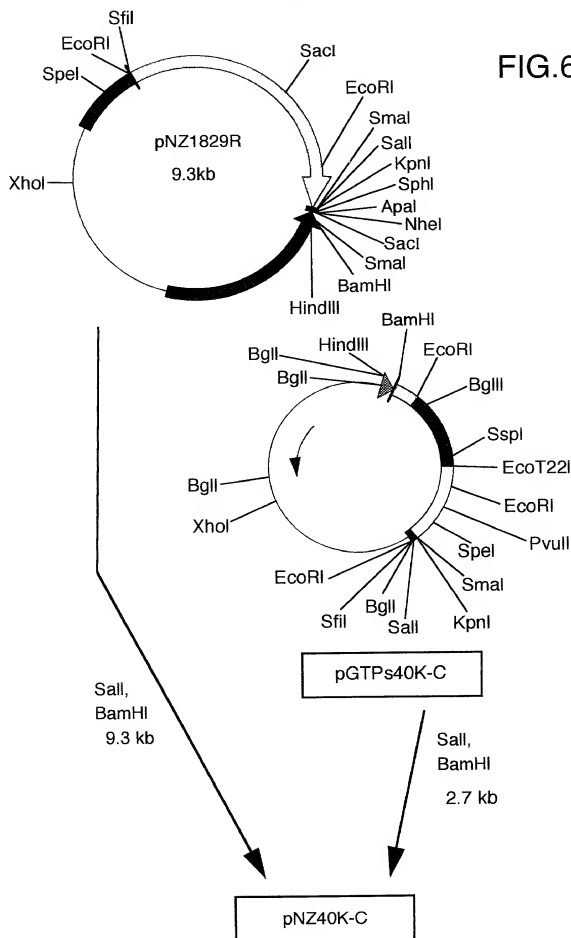
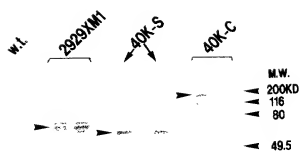
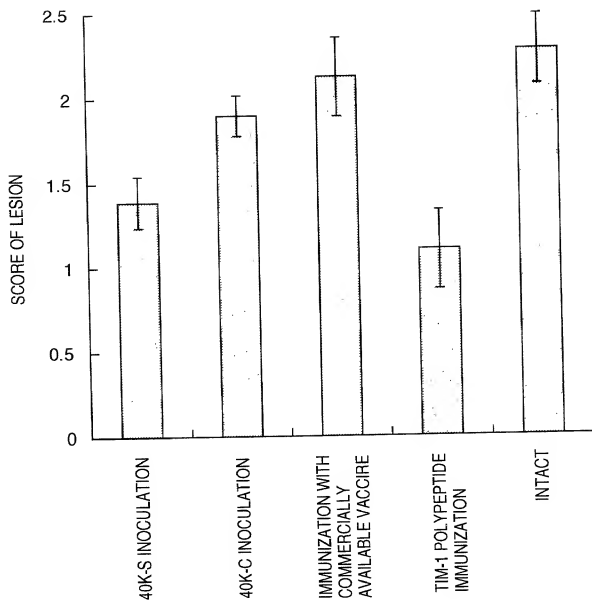


FIG.7



8/8

FIG.8



# Declaration for U.S. Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

(Insert Title) "NOVEL FUSION PROTEIN, GENE THEREOF, RECOMBINANT VECTOR BEARING

THE GENE AND RECOMBINANT VIRUS AS WELL AS USE THEREOF"

the specification of which is attached hereto unless the following is checked:

was filed on March 28, 1997 as United States Application Number or PCT International

☒ Application Number PCT/JP97/01084 and was amended on \_\_\_\_\_  
(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 (a) - (d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

(List prior foreign applications. See note A on back of this page)	<u>08-103548</u>	<u>Japan</u>	<u>29 March, 1996</u>	Priority Claimed <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
	(Number)	(Country)	(Day/Month/Year Filed)	
	(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No
	(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No
	(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No

(See note B on back of this page)

☐ See attached list for additional prior foreign applications

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of the application:

(List Prior U.S. Applications)	(Application Serial Number)	(Filing Date)	(Status) (patented, pending, abandoned)
	(Application Serial Number)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Whereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the United States Code, § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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JAPAN

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Post Office Address c/o Sogokaihatu Centa, NIPPON ZEON CO., LTD., 1-2-1, Yako,  
Kawasaki-Ku, Kawasaki-Shi, KANAGAWA 210 JAPAN

Full name of third inventor (given name, family name) Noboru YANAGIDA  
Inventor's Signature Noboru Yanagida Date 30/9/1998  
Residence Kawasaki-Shi, KANAGAWA JAPAN Citizenship Japan  
Post Office Address c/o Sogokaihatu Centa, NIPPON ZEON CO., LTD., 1-2-1, Yako,  
Kawasaki-Ku, Kawasaki-Shi, KANAGAWA 210 JAPAN

Full name of fourth inventor (given name, family name) \_\_\_\_\_  
Inventor's Signature \_\_\_\_\_ Date \_\_\_\_\_  
Residence \_\_\_\_\_ Citizenship \_\_\_\_\_  
Post Office Address \_\_\_\_\_

Full name of fifth inventor (given name, family name) \_\_\_\_\_  
Inventor's Signature \_\_\_\_\_ Date \_\_\_\_\_  
Residence \_\_\_\_\_ Citizenship \_\_\_\_\_  
Post Office Address \_\_\_\_\_

Full name of sixth inventor (given name, family name) \_\_\_\_\_  
Inventor's Signature \_\_\_\_\_ Date \_\_\_\_\_  
Residence \_\_\_\_\_ Citizenship \_\_\_\_\_  
Post Office Address \_\_\_\_\_

Full name of seventh inventor (given name, family name) \_\_\_\_\_  
Inventor's Signature \_\_\_\_\_ Date \_\_\_\_\_  
Residence \_\_\_\_\_ Citizenship \_\_\_\_\_  
Post Office Address \_\_\_\_\_

Full name of eighth inventor (given name, family name) \_\_\_\_\_  
Inventor's Signature \_\_\_\_\_ Date \_\_\_\_\_  
Residence \_\_\_\_\_ Citizenship \_\_\_\_\_  
Post Office Address \_\_\_\_\_